

Sorting by pools

Master

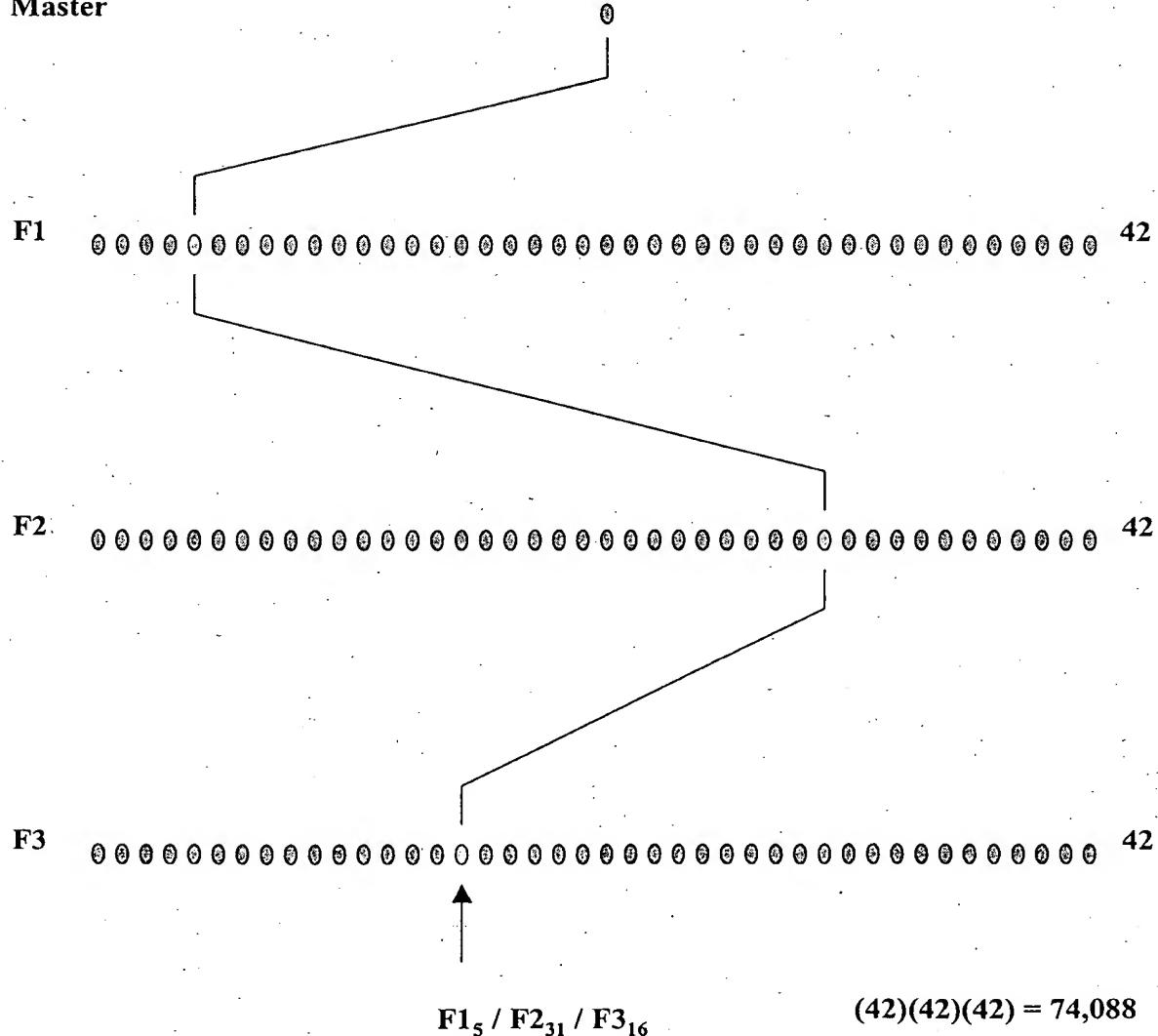


FIGURE 1

Sorting by pools: Decreasing pool diversities

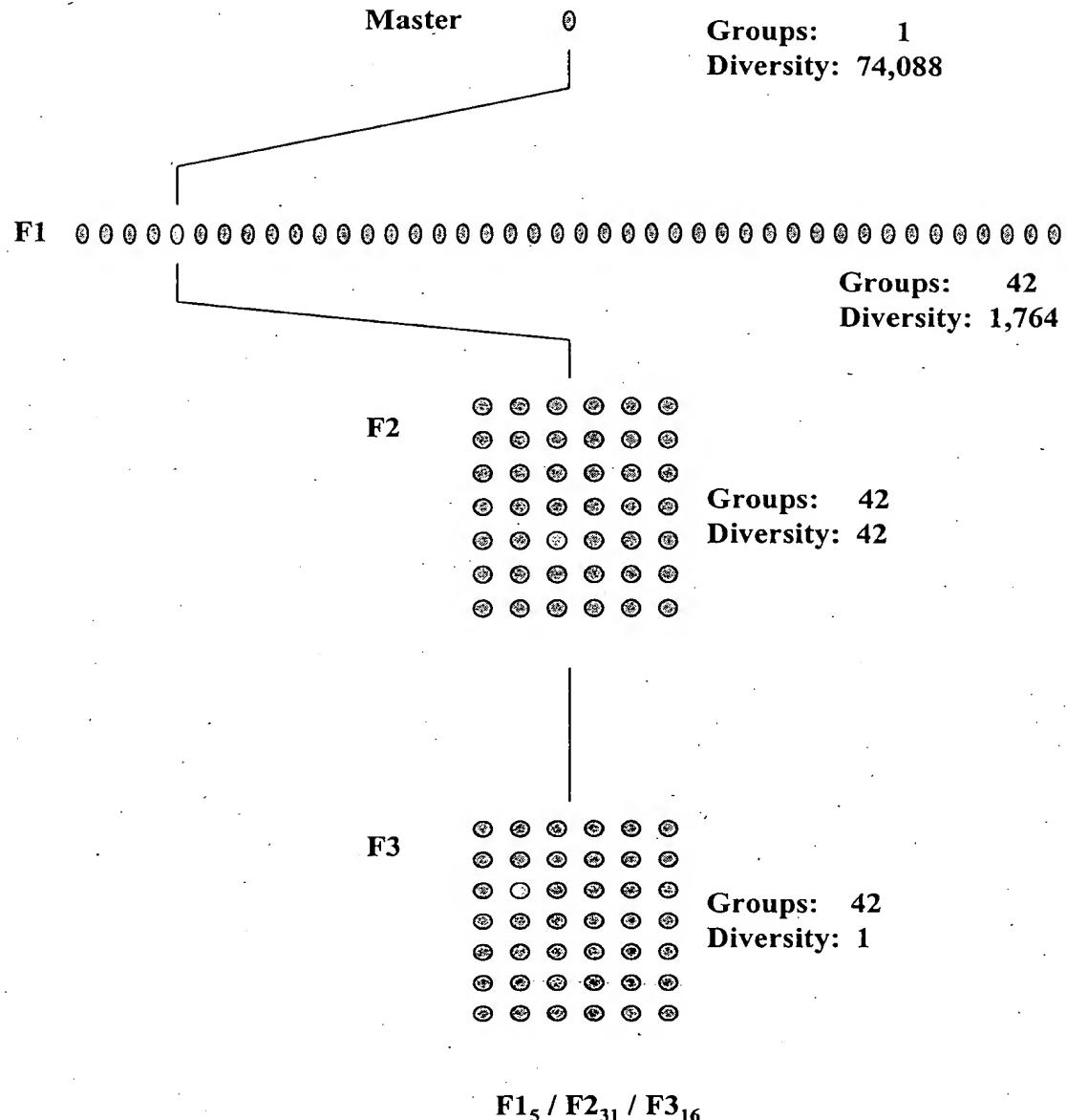


FIGURE 2

Applicant: Ault-Riche *et al.*

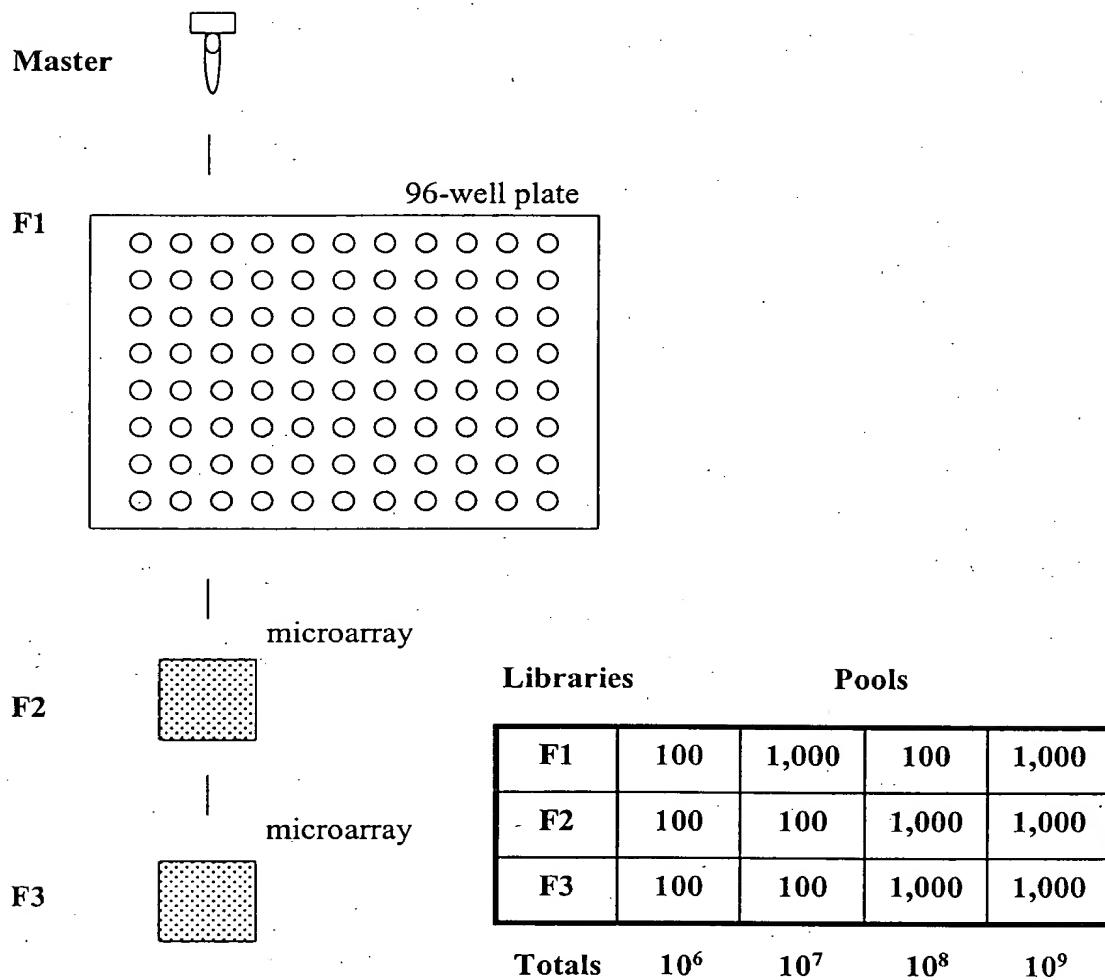
DKT. No. 25885-1751

Priority claimed to 60/219,183

For: COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Sorting by pools: Screening large diversity libraries

TOTAL DIVERSITY

**FIGURE 3**

Searching a mutation library

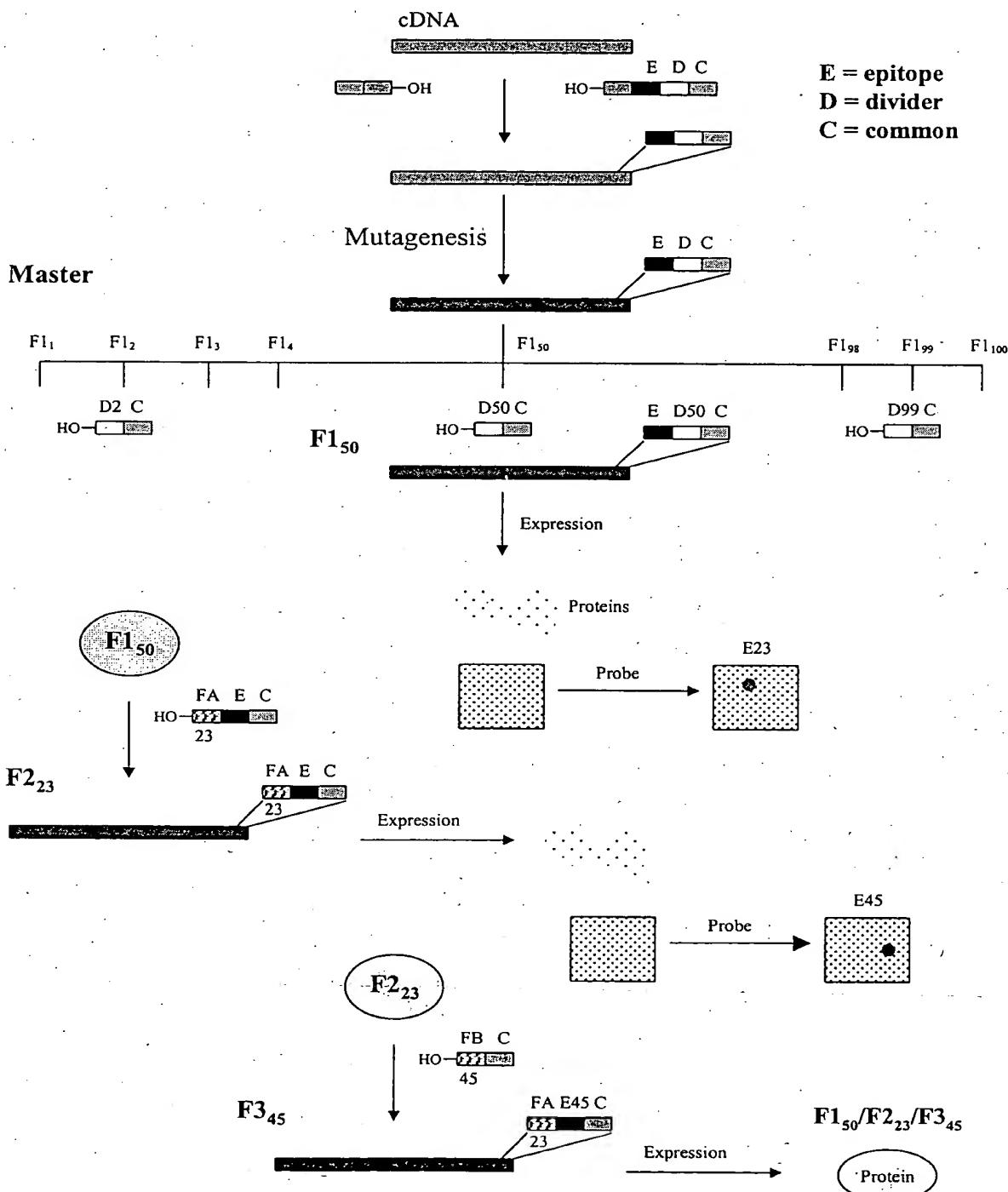


FIGURE 4

Applicant: Ault-Riche *et al.*

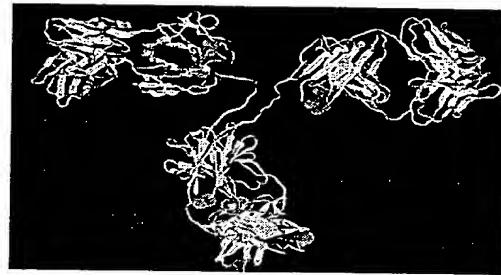
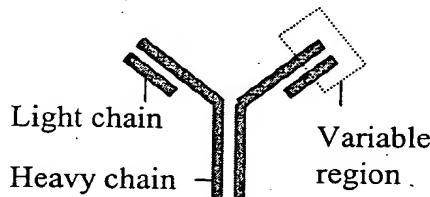
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Making a recombinant antibody library

Basic antibody structure



Spleen cells or PBLs



mRNA



cDNA

V_HV_LV_H

Linker

V_L

Expression

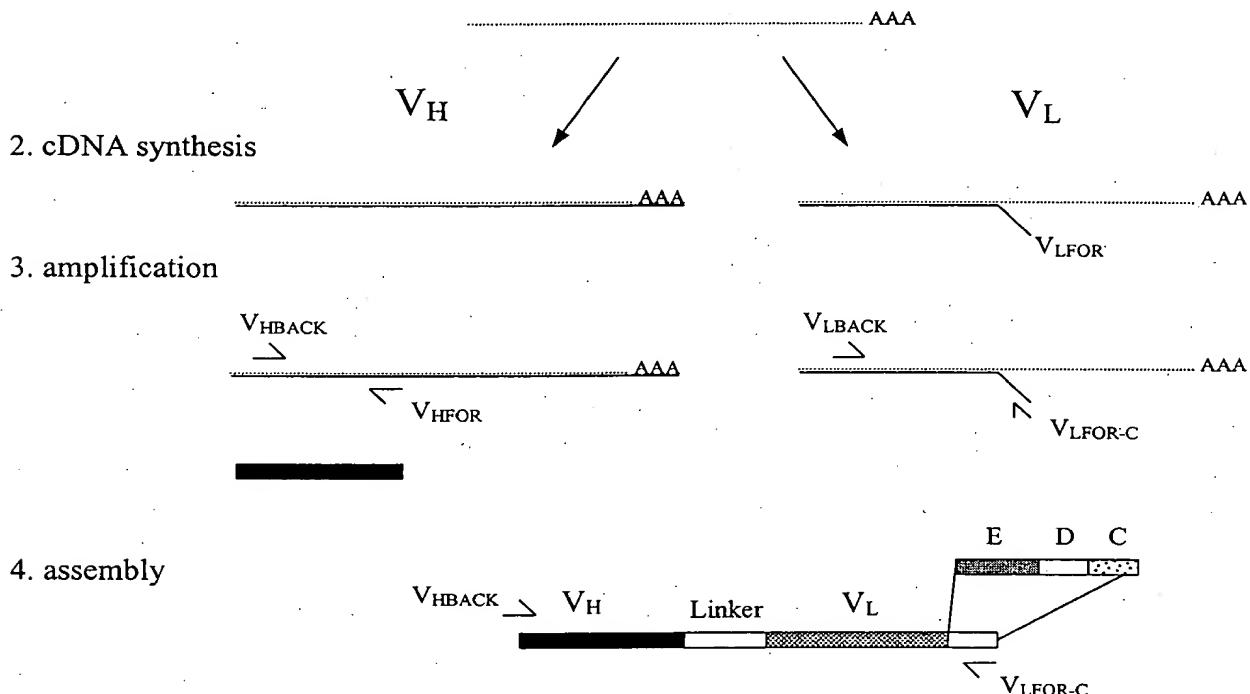


Antibodies

FIGURE 5

Creating the master antibody library: Primer incorporation

- #### 1. mRNA purification from spleen or PBLs

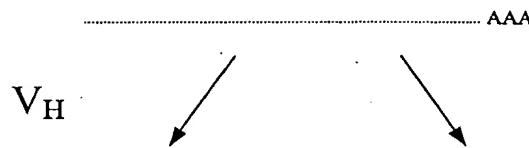


V_H Primers		V_L Primers	
Oligo dT	HO-TTTTTTTT(T) _n	V_{LFOR}	J _{kappa} for E D C
	3' 5'		3' 5'
V_{HBACK}	V _H back	V_{LBACK}	V _{kappa} back
	5' OH		5' OH
V_{HFOR}	J _H for	V_{LFOR-C}	C
	HO-  3'		HO-  3'

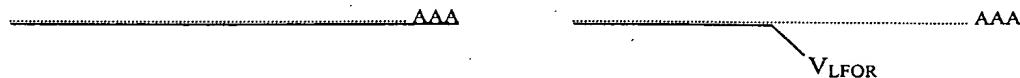
FIGURE 6

Creating the master antibody library: Linker addition

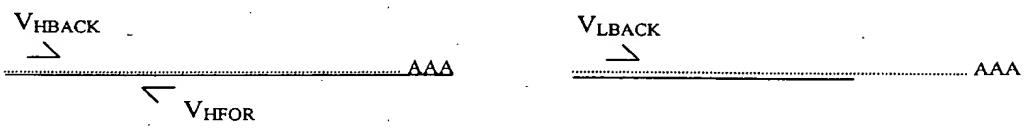
1. mRNA purification from spleen or PBLs



2. cDNA synthesis



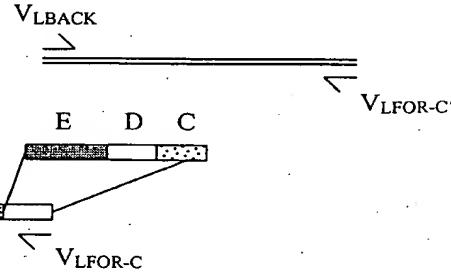
3. amplification



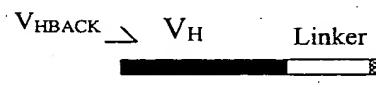
Digest end and mix with $V_{Linkers}$



Ligate and amplify



4. assembly



V_H Primers

Oligo dT HO-TTTTTTTT(T)_n
 3' 5'

V_{HBACK} V_H back-OH
 5' 3'

V_{HFOR} J_H for-OH
 3' 5'

V_L Primers

V_{LFOR} J_{kappa} for-OH
 3' 5'

V_{LBACK} V_{kappa} back-OH
 5' 3'

$V_{Linkers}$ J_{kappa} for-E-D-C-OH
 3' 5'

V_{LFOR-C} C-HO-OH
 3' 5'

FIGURE 7

Searching a recombinant antibody library

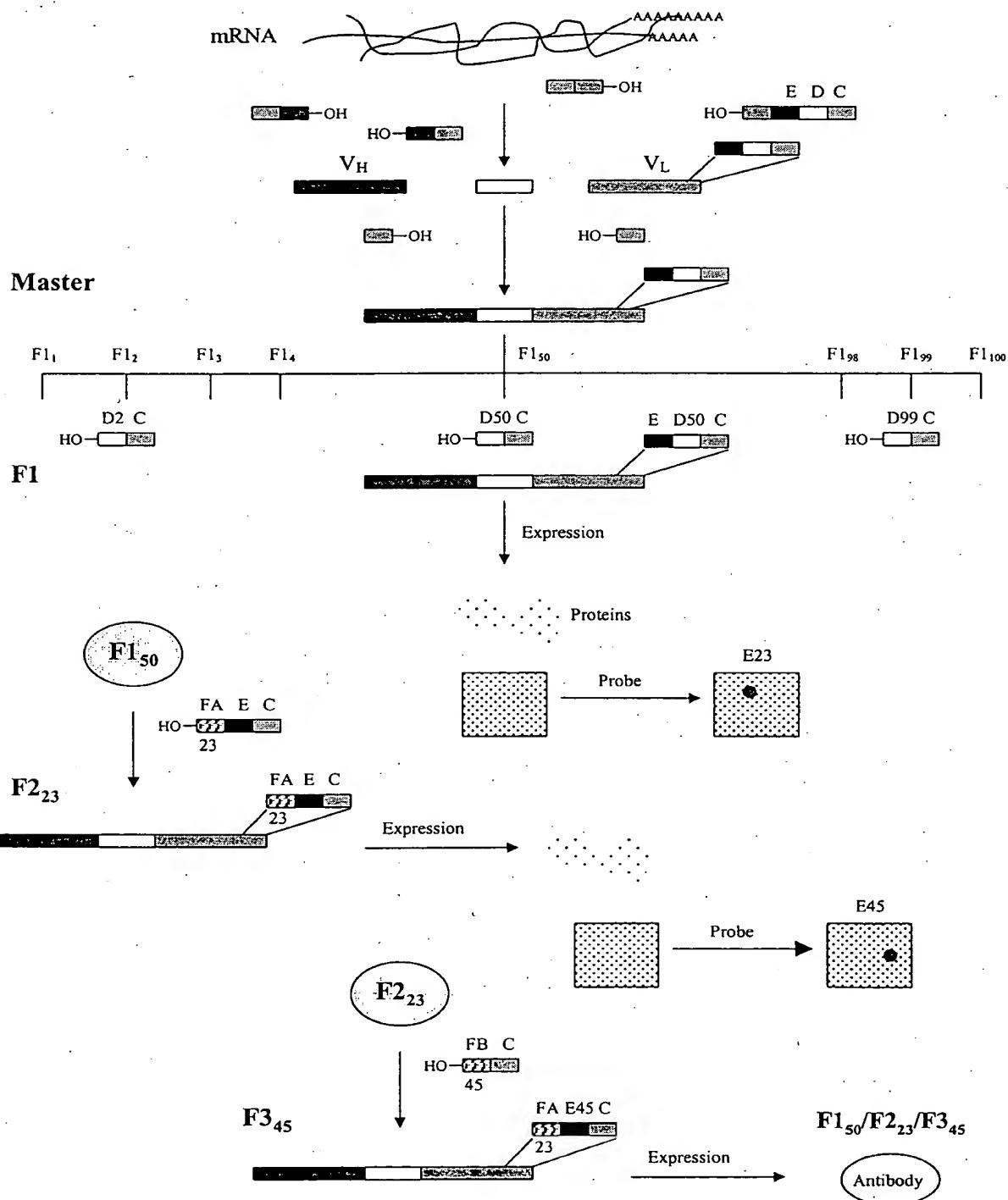
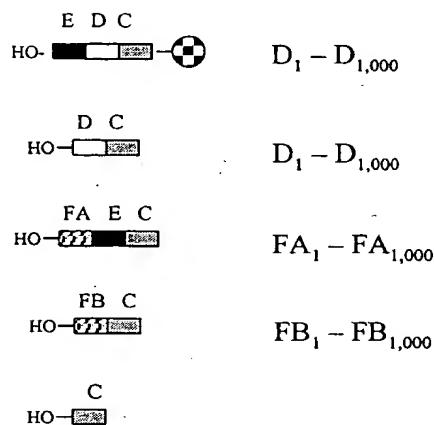


FIGURE 8

Physical elements to include in the kits and combinations

- *Anti-tag Arrays™*

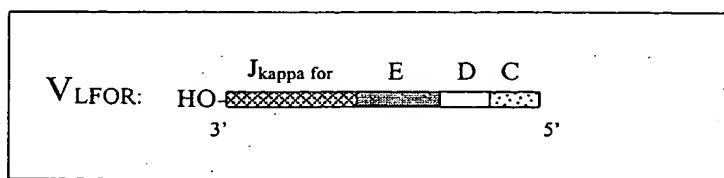
- Primer sets



- Readers
 - Software

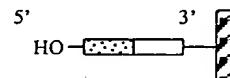
FIGURE 9

Making the V_{LFOR} primers: Solid phase synthesis

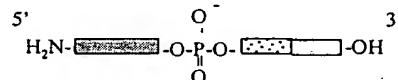


J_{kappa} for Epitope D Common

- ### 1. Synthesize oligo on solid support



- ## 2. Add aminolink prior to cleavage



- ### 3. Couple to tosyl activated magnetic beads



4. Extend by hybridizing with DNA patch and ligating

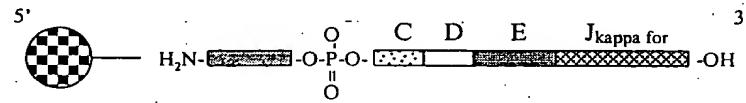
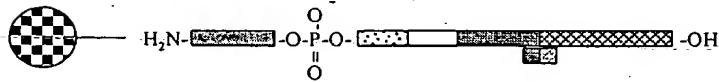
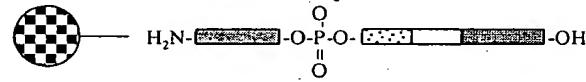
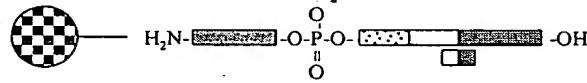
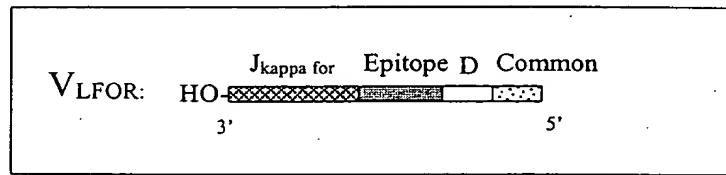


FIGURE 10

Making the V_{LFOR} primers: Overlapping hybridization



- Synthesize 4,028 different oligos:
(26 for J_{kappa} for; 2,000 for Epitope; 2,000 for D; 2 for Common)

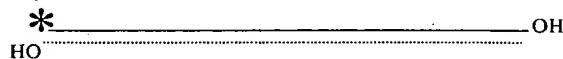
2. Assemble oligos for + and - strands of the different regions



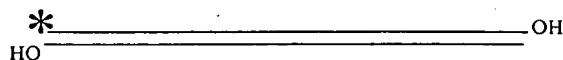
3. Ligase the assembled oligos



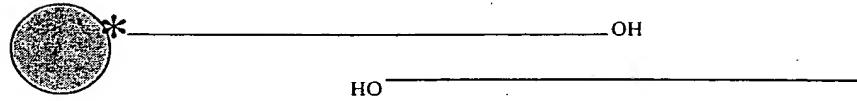
4. 1st strand synthesis with biotinylated primer



- 2nd strand synthesis with non-biotinylated primer



6. Bind to avidin coated magnetic beads and then denature



7. Purify non-biotinylated ssDNA

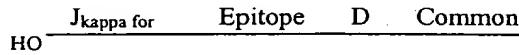


FIGURE 11

Building the collection of antibody/tag pairs: Hybridoma screening

HYBRIDOMA SCREENING

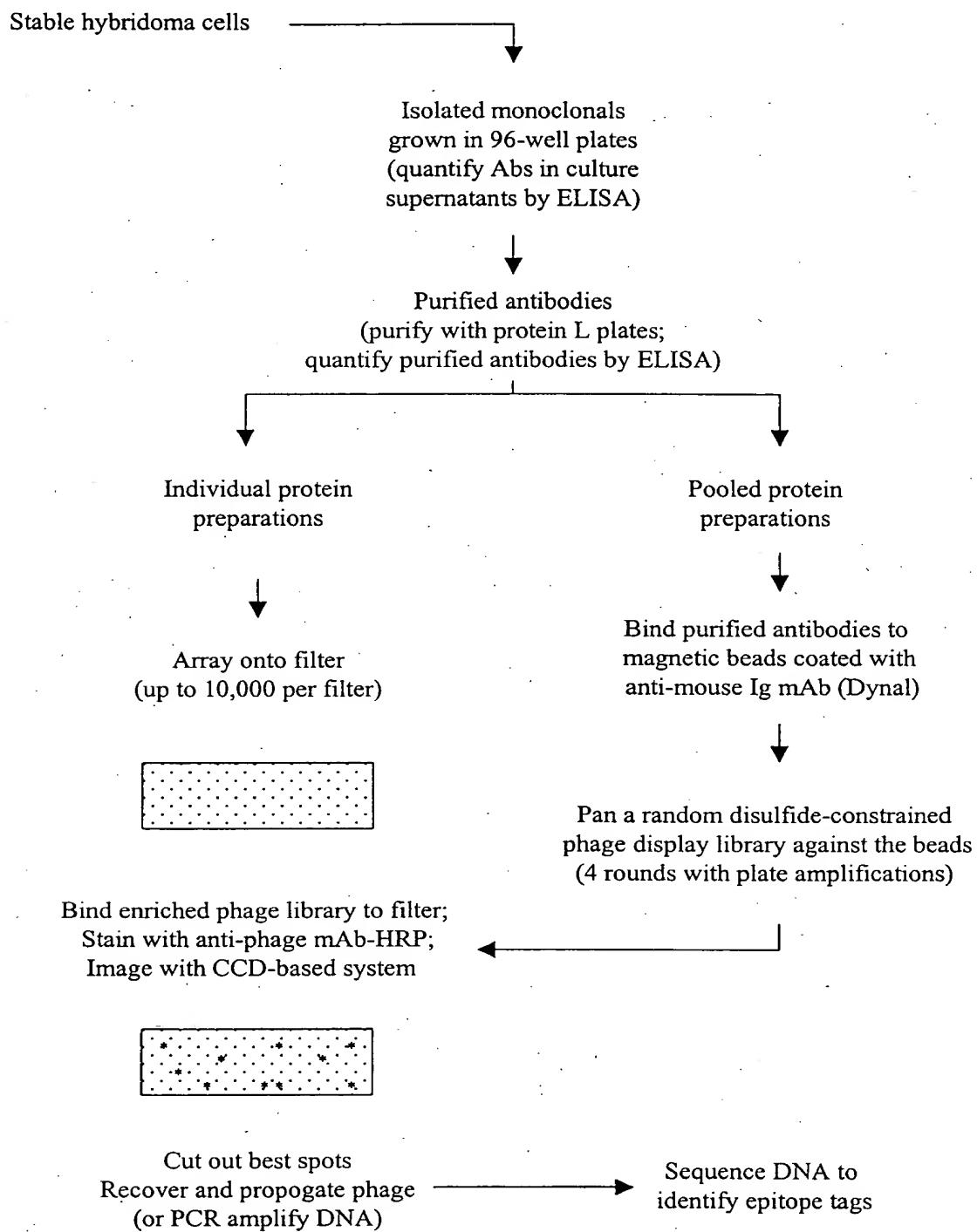


FIGURE 12

FIGURE 13A**TABLE 3** Primers for PCR Amplification of Human Antibody Variable Regions (V genes)**1. V gene primary PCR****A. Human VH back primers (sense)**

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAO GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVk1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuVk2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACC CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACC ACA CTC ACC CAG TCT CC-3'
HuVk6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuVλ1BACK	5'-CAG TCT GTG TTG ACC CAG CCG CC-3'
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT CC-3'
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJκ1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJκ2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJκ3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJκ4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJκ5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J lambda forward primers (anti-sense)

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

continues

FIGURE 13B

TABLE 3 *Continued*

2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

	FR4 heavy	linker
RHuJH1-2	5'-GC ACC CTG GTC ACC GTC TCC'ICA GGT GG-3'	
RHuJH3	5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'	
RHuJH4-5	5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'	
RHuJH6	5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'	

F. Reverse Vk for scFv linker (anti-sense)

	FR1 light	linker
RHuVk1aBACKFv	5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'	
RHuVk2aBACKFv	5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'	
RHuVk3aBACKFv	5'-GG AGA CTG CGT CAA CAC ATT TTC CGA TCC GCC-3'	
RHuVk4aBACKFv	5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'	
RHuVk5aBACKFv	5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'	
RHuVk6aBACKFv	5'-GG AGA CTG AGT CAG CAC ATT TTC CGA TCC GCC-3'	

F. Reverse Vλ for scFv linker (anti-sense)

	FR1 light	linker
RHuVλBACK1Fv	5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'	
RHuVλBACK2Fv	5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'	
RHuVλBACK3aFv	5'-GG TGG CTG AGT CAG CAC ATA CGA CGA TCC GCC ACC GCC AGA G-3'	
RHuVλBACK3bFv	5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'	
RHuVλBACK4Fv	5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'	
RHuVλBACK5Fv	5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'	
RHuVλBACK6Fv	5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'	

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi) primers (sense)

HuVH1aBACKSfi	FR1 heavy
5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CCG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'	
HuVH2aBACKSfi	
5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CCG</u> <u>GCC</u> ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'	
HuVH3aBACKSfi	
5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CCG</u> <u>GCC</u> ATG GCC GAG GTG CAG CTG GTG CAG TCT GG-3'	
HuVH4aBACKSfi	
5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CCG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'	
HuVH5aBACKSfi	
5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CCG</u> <u>GCC</u> ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'	
HuVH6aBACKSfi	
5'-GTC CTC GCA ACT <u>GCG</u> <u>GCC</u> CAG <u>CCG</u> <u>GCC</u> ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'	

H. Human J kappa forward (Not) primers (anti-sense)

HuJk1PORNot	FR4 Light
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACG TTT GAT TTC CAC CTT GGT CCC-3'	
HuJk2PORNot	
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACG TTT GAT CTC CAC CTT GGT CCC-3'	

H. Human J kappa forward (Not) primers (anti-sense) (Continued)

HuJk3PORNot	FR4 light
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACG TTT GAT ATC CAC TTT GGT CCC-3'	
HuJk4PORNot	
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACG TTT GAT CTC CAC CTT GGT CCC-3'	
HuJk5PORNot	
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACG TTT AAT CTC CAG TCG TGT CCC-3'	

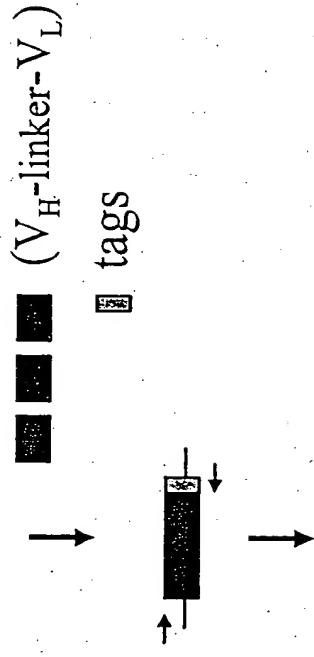
I. Human J lambda forward (Not) primers (anti-sense)

Hu J11FORNOT	FR4 light
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACC TAG GAC GGT GAC CTT GGT CCC-3'	
Hu J12-3FORNOT	
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACC TAG GAC GGT CAG CTT GGT CCC-3'	
Hu J14-5FORNOT	
5'-GAG TCA TTC TCG ACT <u>TGC</u> <u>GGC</u> <u>CGC</u> ACC TAA AAC GGT GAG CTG GGT CCC-3'	

*Recognition site for restriction enzyme is underlined.

step I

Tag and assemble immunoglobulin genes

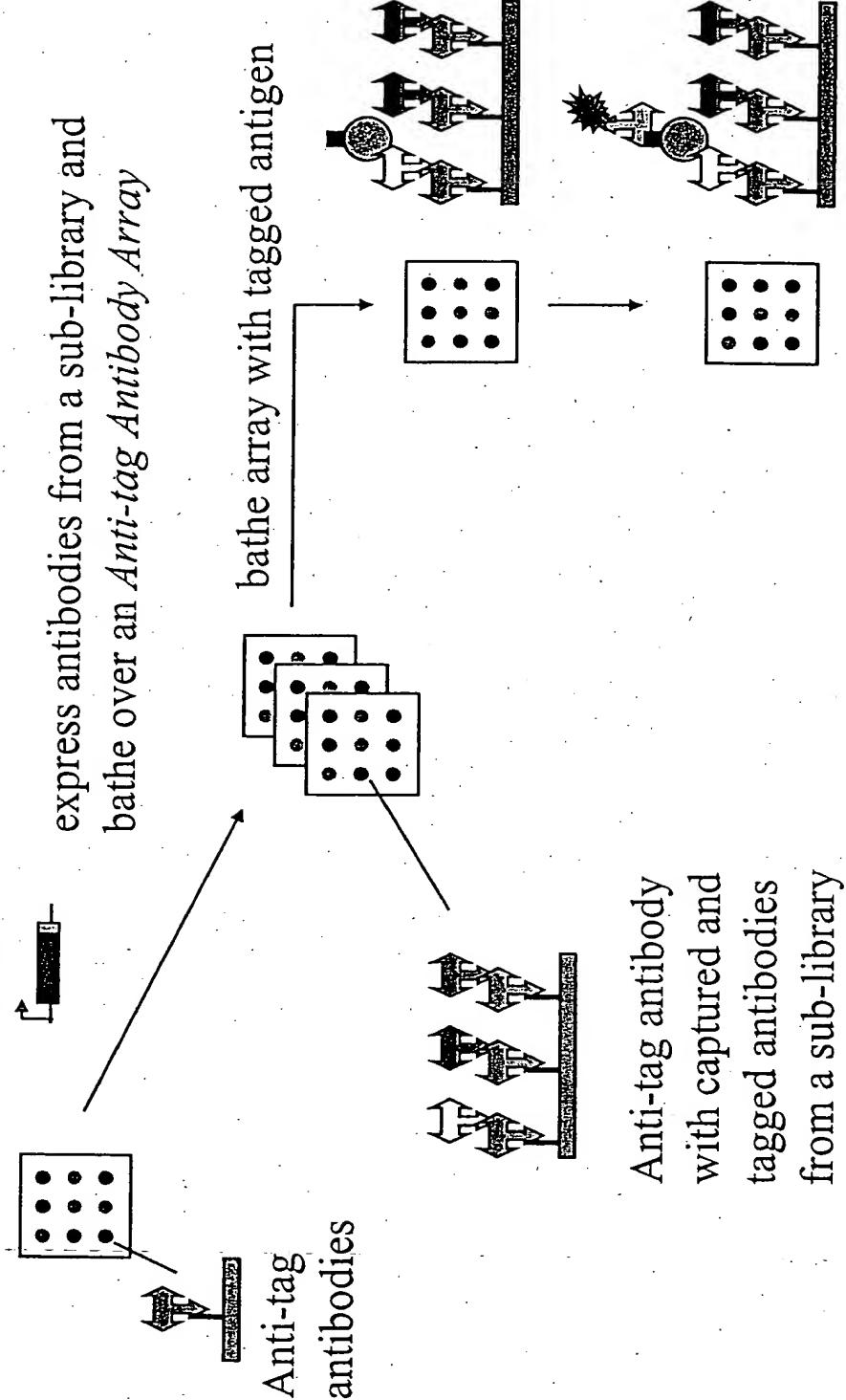


Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers



1,000 sub-libraries

step II



ID spot containing the antigen
with a labeled developing Ab

FIGURE 14B

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Sheet 16 of 23
Applicant: Aut-Ridge et al.
DKT. No. 25885-1751
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F C D T F D O " D E T O F G G G O

step III

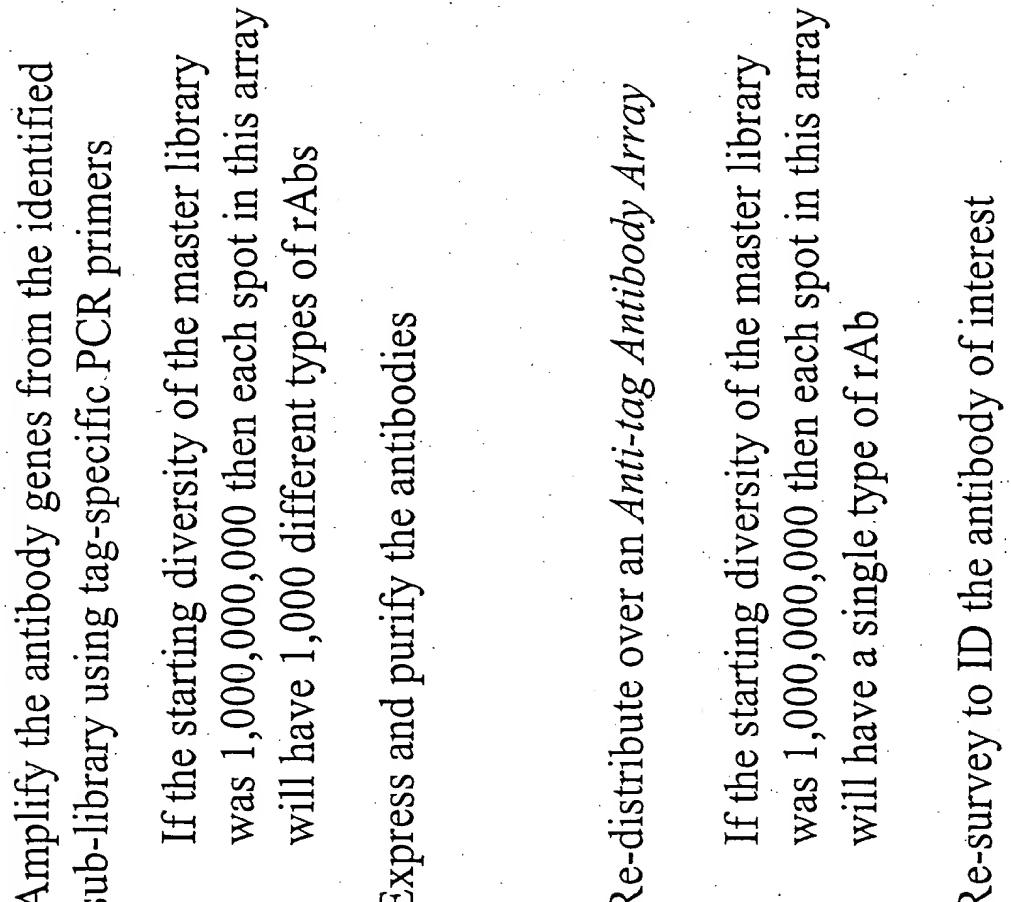
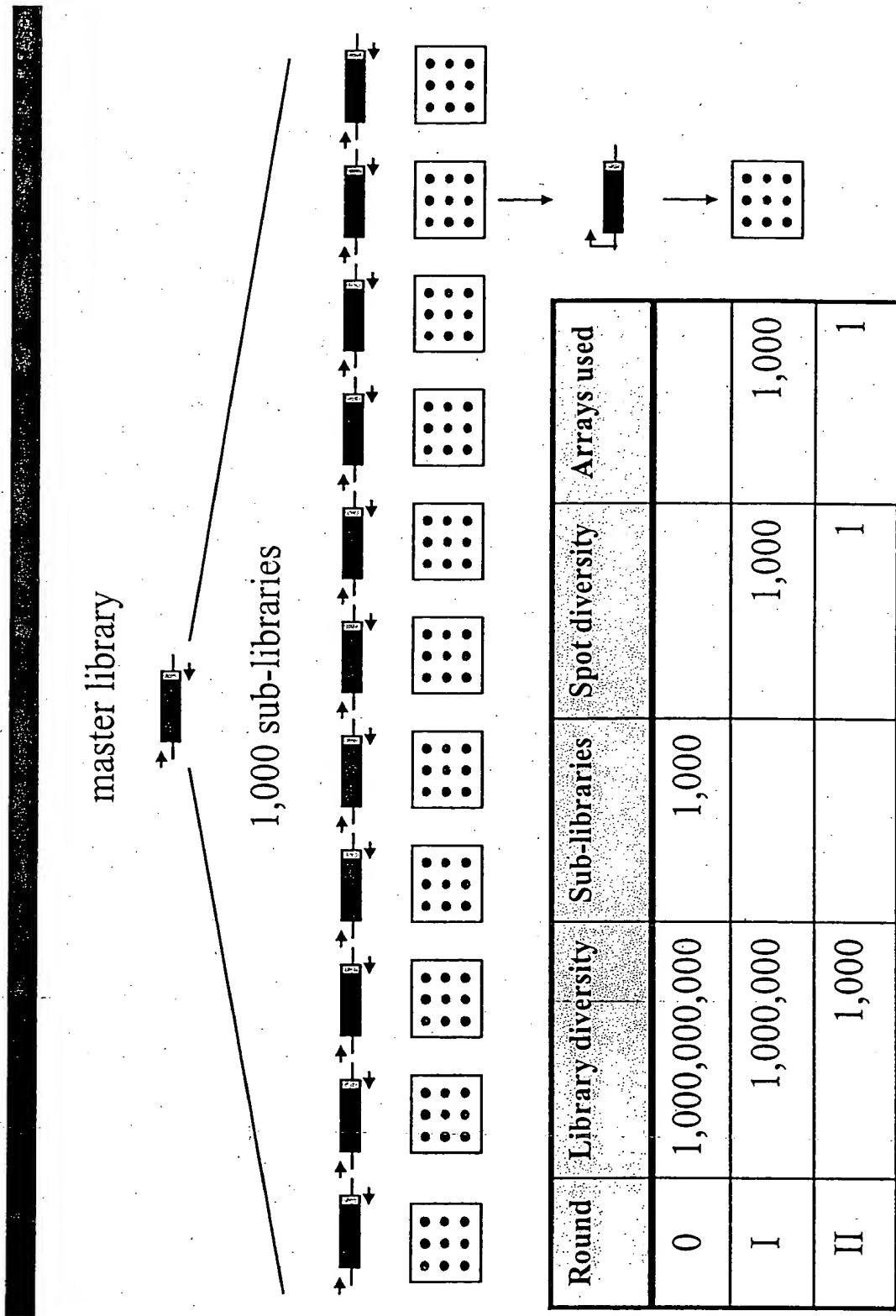


FIGURE 14C

FIGURE 14D

summary



ER EHRMAN WHITE & MCGULIFFE LLP
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Applicant: Aut-Riche et al.

FIGURE 14D

- Modification searches

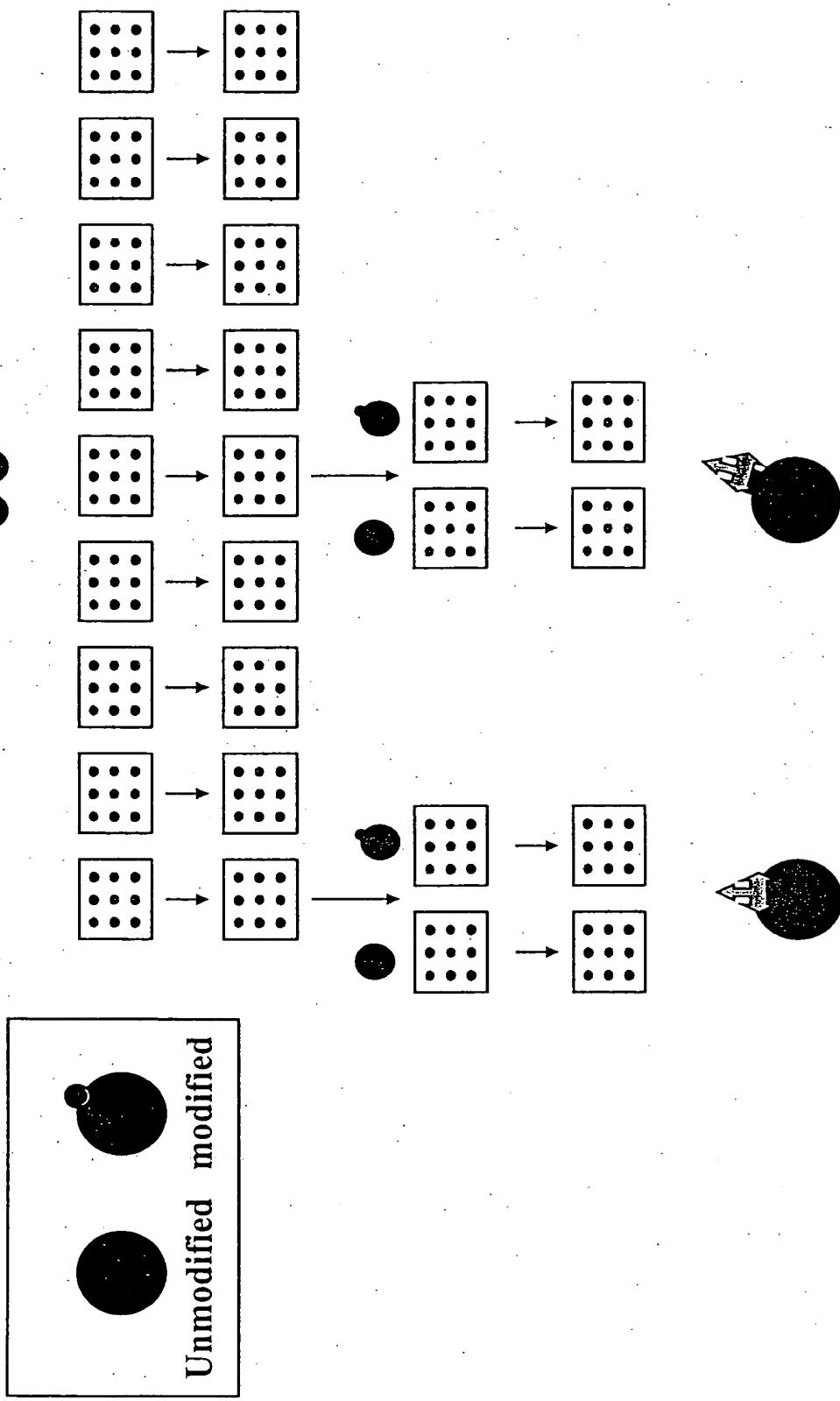
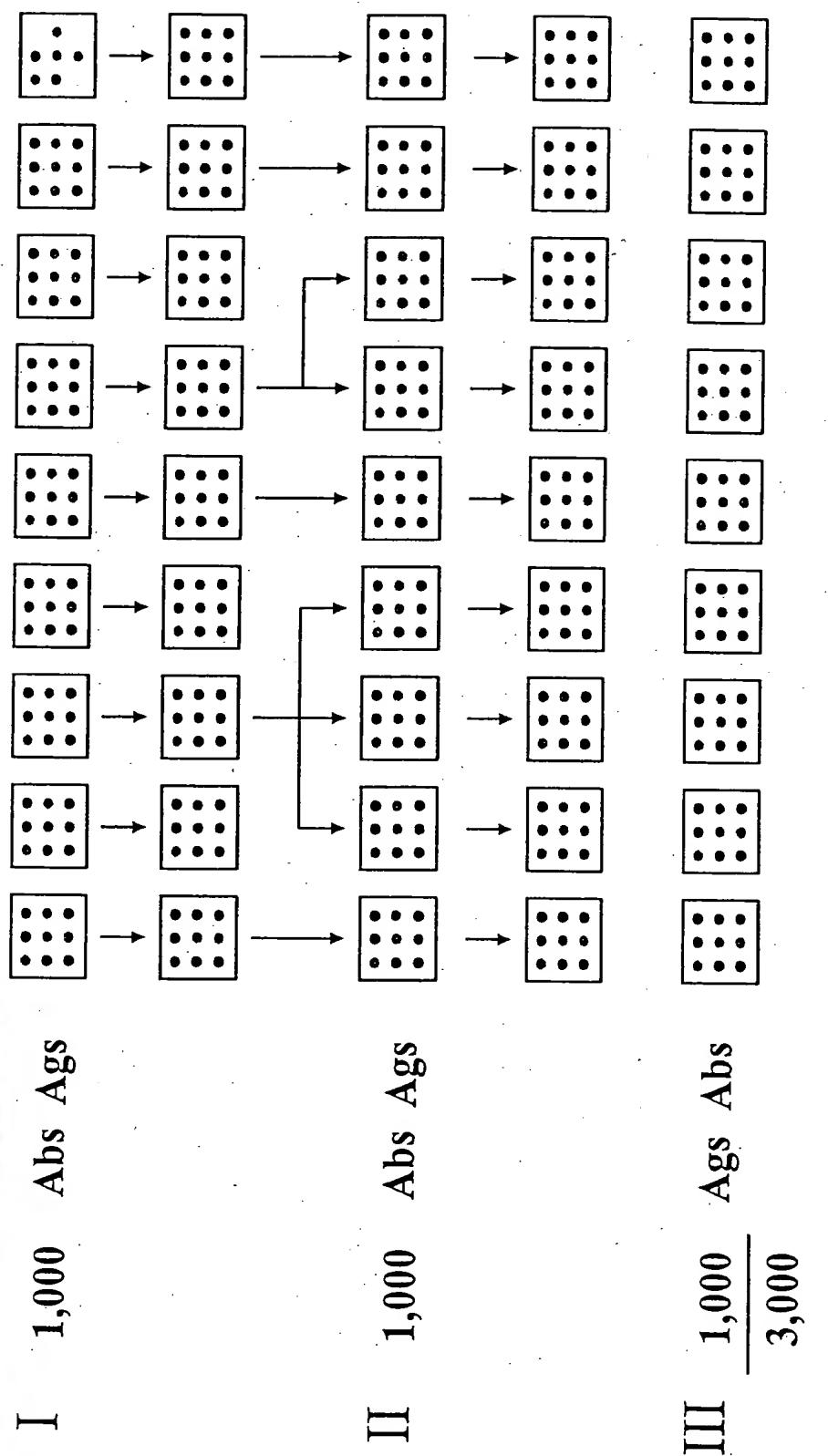


FIGURE 15

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DKT. No. 25885-1751
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Round Arrays Bait Probe

I 1,000 Abs Ags



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AND HIGH THROUGHPUT SCREENING

FIGURE 16

3 Arrays per Ag

Enzyme engineering

Patent 2007016560

Natural gene(s) Error-prone PCR
or Gene Shuffling

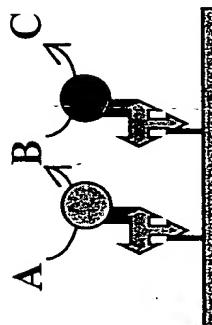


- tag the gene to be mutated
- mutate genes and create sub-libraries

- distribute mutants over arrays

- probe the arrays with labeled substrates

Spots can contain mixtures of enzymes
for detection or pathway engineering

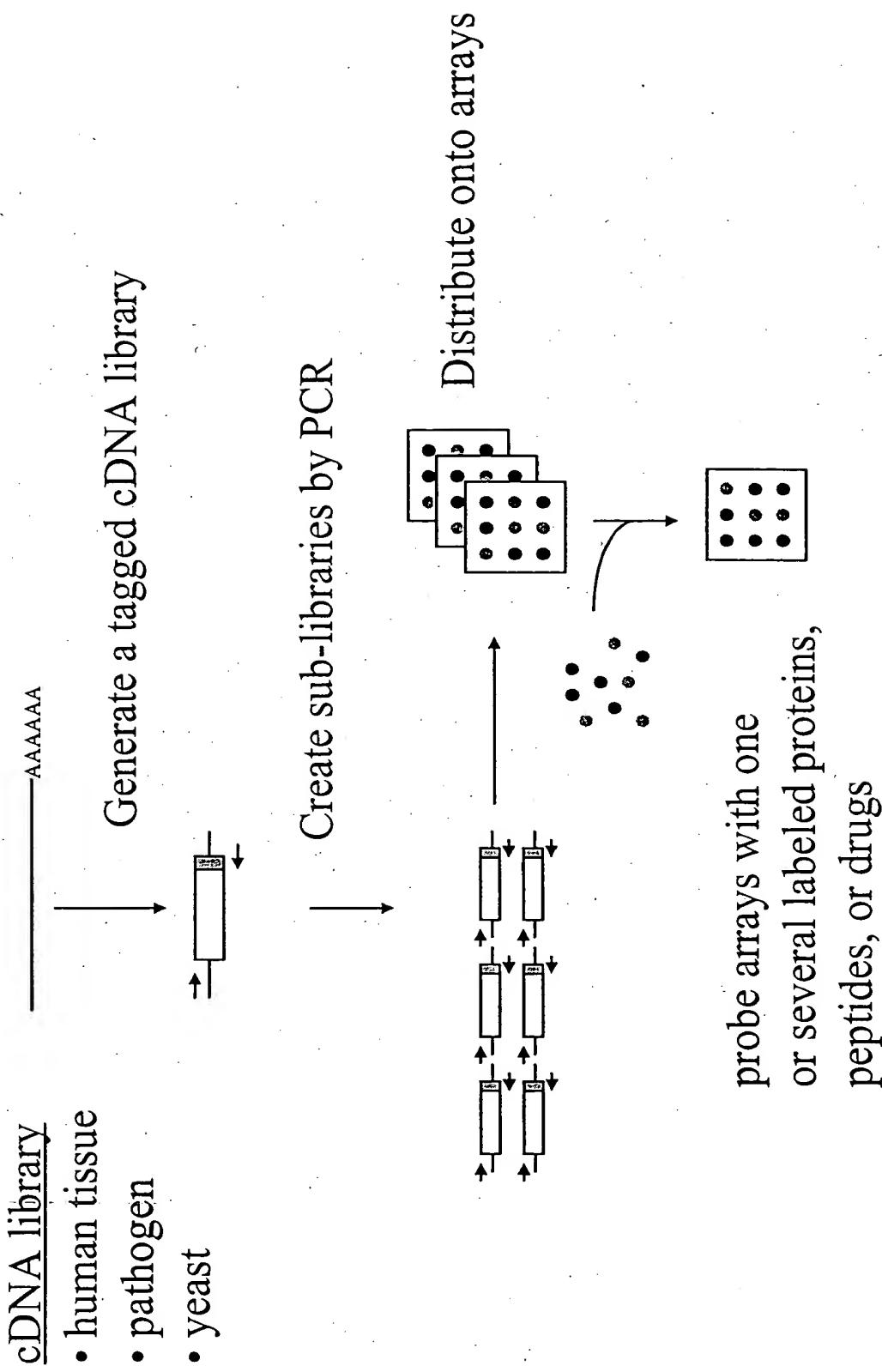


HE ERMAN WHITE & MGAULIFFE LLP
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Application: Aut-Riche et al.
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FIGURE 17

Protein interaction mapping

FIGURE 18 - DATA FIGURE



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FIGURE 18

FIGURE 19

